UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/880,149	06/14/2001	John H. Kenten	2757-5	7213

7590 10/03/2002

Nixon & Vanderhye, P.C.
8th Floor
1100 North Glebe Road
Arlington, VA 22201-4714

EXAMINER
WINKLER, ULRIKE

ART UNIT	PAPER NUMBER
1648	9

DATE MAILED: 10/03/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application N .	Applicant(s)	
	09/880,149	KENTEN ET AL.	
	Examiner	Art Unit	
	Ulrike Winkler, Ph.D.	1648	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *24-30,36,37 and 40* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *24-30,36, 37 and 40* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *1 and 4* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Specification

Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

Sequence listing

Applicant's CRF and paper sequence listing have been entered.

Information Disclosure Statement

An initialed and dated copy of Applicant's IDS form 1449, Paper Nos. 1 and 4, are attached to the instant Office Action.

Drawings

The drawings have been approved by the Draftsperson.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 24-30, 36, 37 and 40 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for ubiquitination of a protein using a compound in a cell lysate assay system, does not reasonably provide enablement for ubiquitination of a compound within a cell (claims 24, 25, 29, 36, 37 and 40) and for the use of the compound as a pharmacological agent in a patient (claims 26-28 and 30). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The factors to be considered in determining whether undue experimentation is required are summarized *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). They include: (1) the nature of the invention, (2) the state of the prior art, (3) the presence or absence of working examples, (4) the amount or direction or guidance presented,(5) the quantity of experimentation necessary, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

The specification does not set forth sufficient teachings to allow one skilled in the art to use the claimed method of reducing the activity of a target protein by directing the protein to the ubiquitin degradation pathway using a compound within a cell or within a patient. (1) the nature of the invention is to bring a target molecule into close proximity to the ubiquitin conjugating enzymes so that the target molecule will be labeled with ubiquitin and subsequently be degraded.

Issues of concern are availability of the compound to the ubiquitination system and the accessibility of the compound to cells within a patient.

(2) the state of the prior art, is such that the N-terminal amino acids that target a protein for rapid degradation in prokaryotic and eucaryotic systems is known (see Varshavsky A., PNAS 1996, table 1, IDS Paper No. 1). The predictability based on the prior art is such that changing the N-terminal amino acid sequence of a protein would target a protein to the ubiquitin conjugating enzymes and once labeled the protein will be degraded. The prior art lacks predictability in regards to the proximity requirements for the enzyme substrate/target site. Removing the first two N-terminal lysines (see Kwon et al., Journal of Biological Chemistry, 1999, page 18137, column 1 paragraph 2, IDS Paper No. 1) slows the rate of ubiquitination of the substrate shows that not only is there an N-terminal amino acid requirement, but proximity of the lysines which are the target for the ubiquitin conjugating enzymes is critical for ubiquitination of a target protein.

(3) the presence or absence of working examples, the specification does not provide any working examples that would indicate the claimed compounds are able to enter a cell or be administered to an animal in such a way that they will be effective at targeting a protein to the ubiquitination pathway. The specification does not provide any evidence that the compound is taken up (absorbed) by a cell. There is no evidence that the compound is not degraded before it will reach the target cell and before it will reach the target protein destined for destruction. The specification provides experimental observation from cell-extracts using rabbit reticulocyte, HeLa cell and Jurkat cell lysate (examples 3 and 4). The specification has shown a compound that comprises a recognition element attached to fluorascein. Here the anti-fluorascin antibody is

the target molecule for ubiquitin. Another compound is a recognition element conjugated to 4-aminophenyl arsenoxid.

The ubiquitination recognition element must be capable of interacting with the enzyme (receptor) of interest E2 or E3 with high specificity. If the binding interaction is not highly specific, the ubiquitination recognition element (ligand) will bind to alternative binding sites that may be present in the preparation. Non-specific binding generally is of lower affinity than specific binding (Matthews J., Fundamentals of receptor, enzyme and transport kinetics, CRC Press, Inc. (1993) pp. 28-30, 121-126). Non-specific binding of proteins to plastic has been measured to be in the range of 1-1.89 * 10^7 M^{-1} (Vallabhajosula et al. Non-specific binding of transferring and lactoferrin to polystyrene culture tubes: role of the radioligand. European Journal of Nuclear Medicine (1983) Vol. 8, No. 5, abstract only). Binding affinity of antibody-antigen binding varies a great deal from below 10^5 M^{-1} to greater than 10^{12} M^{-1}, for comparison trypsin binding to its substrate is 1.25 x 10^4 M^{-1} , for antibodies affinities of less than 10^6 M^{-1} would provide a weak signal (Harlow et al. In Antibodies: A laboratory manual, ed. Harlow et al. (1988)). It is not clear that a binding affinity of 10^2 M^{-1} would have a particular specificity for E2 or E3 in relation to any other protein in the cytosol of a cell a binding affinity of 10^2 M^{-1} is very low and it is not clear that this would be sufficient to bind a recognition element with sufficient strength as to guide a target protein to the ubiquitination system.

Most of the substances that must cross membranes are not freely permeable to the membrane structure. Therefore, specific transport systems are present that not only facilitate the flow of materials from one side of the membrane to the other but also regulate the flow. Transport systems can be classified into two broad categories. Those requiring the input of

energy are called active transport systems. Those that do not require the input of energy are called passive transport systems (Matthews J., Fundamentals of receptor, enzyme and transport kinetics, CRC Press, Inc. (1993) pp. 28-30, 121-126). Lysosomes are responsible for degrading exogenous and endogenous proteins which enter the cells by endocytosis and are degraded within endosomes or rerouted to the lysosome for degradation (Krishnamoorthy et al. Chapter 3: Peptide metabolism by gastric, pancreatic and lysosomal proteinase, In: Peptide Based Drug Design, ed. Taylor et al. (1995) American Chemical Society, pp. 47-68, see p58-60). Proteins sequestered by a nonselective bulk process within the lysosomes turn over with an apparent half-life of about 8 minutes. The liver is a potential site for removal of macromolecules such as peptides and proteins following oral delivery. Because the liver is well-perfused and comprises several cell types, including hepatocytes, Kupffer cells, and endothelial cells it is an important organ for protein metabolism. Uptake of peptides and proteins from plasma by hepatocytes occurs by two distinct, yet not entirely separable processes: receptor-mediated endocytosis and nonselective pinocytosis. In receptor-mediated endocytosis, plasma-derived proteins become internalized postbinding by hepatocyte receptor proteins located within the plasma membrane. The receptors can be of "functional" and "clearance" types, and clearance of a protein can be accentuated if a protein binds to both (Krishnamoorthy et al., *supra*, p. 61). The ubiquitin pathway of degradation is located within the cytosol and is highly regulated. (Krishnamoorthy et al., *supra*, p. 61). The specification has provided no evidence that would indicate the contemplated compounds reach the cytosol where the ubiquitinylation enzymes are present. This is essential because if the compounds become degraded before reaching their target by being

taken up via the endosomal pathway the compounds will not reach their target destination (cytosol) and will not be able to facilitate the ubiquitination of the target protein.

Characteristics of a compound's activity *in vitro* using purified or partially purified components generally differs significantly with the compound when used on a whole cell. Additionally, cultured cell lines generally differ significantly from *in vivo* animal models. To be effective within a patient the compound must be delivered into the circulation in a sufficient concentration and for a sufficient period of time. *In vitro* assays cannot duplicate the complex conditions of *in vivo* therapy. In the *in vitro* cell-free assays, the compound is in constant contact with ubiquitination machinery. This is not the case *in vivo*, where exposure to the target may be delayed or inadequate. In addition, variables such as biological stability, half-life or clearance from the blood are important parameters in achieving successful therapy with the compound. The composition may be inactivated *in vivo* before producing a sufficient effect, for example, by proteolytic degradation or immunological activation. In addition, the composition may not otherwise reach the target because of its inability to penetrate tissues or cells where its activity is to be exerted, may be absorbed by fluids, cells and tissues where the composition has no effect, circulation into the target area may be insufficient to carry the composition and a large enough local concentration may not be established.

(4) the amount or direction or guidance presented, the specification provides evidence of using specific ubiquitination recognition elements which are chemically cross-linked with a target protein and show an increase in ubiquitination of the target protein under *in vitro* cell free experimental conditions. The specification does not provide sufficient guidance to allow one skilled in the art to use the claimed method of "activating" the ubiquitination activity directed to

a target protein inside a eukaryotic cell which is the essential feature of the claimed invention. The specification does not provide teachings to establish effective dosages or methods of administration of activating compounds. The specification does not provide any teaching as to how to administer the activating compounds to effectively treat an animal or human. No working examples are provided which would provide sufficient guidance to allow one skilled in the art to practice the embodiments of the invention with a reasonable expectation of success.

There is insufficient guidance and objective evidence in the art that would indicate a compound will be able to enter a cell and be effective *in vivo*, i.e. in an individual. Those of skill in the art recognize *in vitro* assays and or cell-cultured based assays are generally useful to observe basic physiological and cellular phenomenon such as screening the effects of potential drugs. However, clinical correlation is generally lacking. The greatly increased complexity of the *in vivo* environment as compared to the very narrowly defined and controlled conditions of an *in vitro* assay, especially a cell free assay, does not permit extrapolation of *in vitro* assays to animal or human efficacy with any reasonable degree of predictability. *In vitro* assays cannot easily assess cell-cell interactions that may be important in a particular pathological state.

For example an *in vivo* vs. *in vitro* model, passaged U-937 human leukemic cells behaved differently when these cells are passage *in vitro* or *in vivo* (see Chomienne et al., Discrepancy between *in vitro* and *in vivo* passaged U-937 human leukemic Cells: Tumerorigenicity and sensitivity to differentiating drugs. In Vivo (1988)). Passageing the U-937 cells in mice resulted in the cells losing the ability to differentiate when exposed to differentiating drugs (see Figure 6). The authors were not able to explain this in dedifferentiation phenomenon for leukemic cells, but it is clear that host factors play an important role, either in selecting pre-existing less

differentiated cells or by inducing modifications in the cells' proliferation/differentiation status (see Chomienne et al., *supra*, p. 286, column 1, 1st paragraph).

(5) the quantity of experimentation necessary, is high as it is not predictable that a compound will be taken up by the cells and that they will be available and active in the cytosolic environment of the cell where the ubiquitination enzymes are found. (6) the relative skill of those in the art is high. (7) the predictability or unpredictability of the art, the predictability based on the prior art is such that changing the N-terminal amino acid sequence of a protein would target a protein to the ubiquitin conjugating enzymes and once labeled these proteins will be degraded. The prior art lacks predictability in regards to the ability of the compounds to traverse the cell membrane and localize to the cytosol as well as being active within the environment of the cytosol. There is lack of predictability in the art as to the effect of a compound *in vivo* versus the effect of the same compound in an *in vitro* assay environment. (8) the breadth of the claims, the specification provides insufficient guidance with regard to the issues of binding affinity to the ubiquitination system, cellular uptake and availability and provides no working examples of *in vivo* experiments which would provide guidance to one skilled in the art. No evidence has been provided which would allow one of skill in the art to predict the efficacy of the claimed of activation compound of the ubiquitination system as treatment method. Without sufficient guidance the applicant is inviting the artisan to perform undue experimentation. Therefore the instant invention is not enabled for the broadly claimed method of activation of ubiquitination, treatment of disease and administering the compound to an animal.

Conclusion

No claims allowed.

The art made of record and not relied upon is considered pertinent to applicant's disclosure.

Sakamota et al. Protacs: Chimeric molecules that target proteins to the Skp1-cullin-Fbox complex for ubiquitination and degradation. Proceedings of the National Academy of Science (2001) pp. 8554-8559.

Deshaies et al. Proteolysis targeting chimeric molecule. Pub. No. US 2002/0068063 A1 (June 6, 2002).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ulrike Winkler, Ph.D. whose telephone number is 703-308-8294. The examiner can normally be reached M-F, 8:30 am - 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James Housel, can be reached at 703-308-4027.

The fax phone numbers for the organization where this application or proceeding is assigned are 703-308-4242 for informal communications use 703-308-4426.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

Ulrike Winkler, Ph.D. 10/1/02